

Transcriptional control of the IL-5 gene by human helper T cells: IL-5 synthesis is regulated independently from IL-2 or IL-4 synthesis

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Background: IL-5 is fundamentally involved in eosinophilic inflammation. Control of IL-5 production may be effective for the management of allergic diseases.

Objective: We aimed to find the transcriptional mechanisms that regulate the IL-5 gene to selectively control IL-5 synthesis. **Methods:** Allergen-specific T-cell clones and T-cell hybridomas were established from the peripheral blood lymphocytes of patients with asthma, and the transcriptional regulation of the IL-5 gene was investigated with transient transfection and electrophoretic mobility shift analysis.

Results: A human IL-5 promoter/enhancer-luciferase gene construct, pIL-5(-511)Luc, was transcribed on activation of IL-5-producing T-cell clones, but not IL-5-nonproducing clones. pIL-5(-511)Luc was transcribed by T-cell hybridomas derived from fusion between IL-5-producing T-cell clones and an IL-5 gene-nonexpressing T-cell line, but not by hybridomas derived from IL-5-nonproducing T-cell clones. IL-5 synthesis was not only induced by T-cell receptor stimulation but also by IL-2 receptor stimulation. Binding of NF-AT, NF- κ B, and AP-1 was induced by T-cell receptor (TcR) stimulation, although there was no significant upregulation of binding by IL-2 stimulation. **Conclusion:** IL-5 synthesis by human helper T cells is regulated at the transcriptional level. A unique transcriptional mechanism distinct from those regulating the IL-2 or IL-4 genes seems to control the IL-5 gene. Selective regulation of IL-5 gene transcription may be useful for treating eosinophilic inflammation. (*J Allergy Clin Immunol* 1999;103:S429-36.)

Key words: IL-5, T cell, gene transcription

Chronic eosinophilic inflammation of the bronchial mucosa has been well recognized to have a role in the pathogenesis of bronchial asthma.¹ Helper T cells and T-cell cytokines, especially IL-5, have been strongly implicated in promoting the local infiltration and activation of eosinophils.² The number of CD4⁺ T cells expressing IL-

Abbreviations used

CEM-8Az:	8-Azaguanine-resistant human transformed T-cell line
Der f:	<i>Dermatophagoides farinae</i>
EMSA:	Electrophoretic mobility shift assay
mRNA:	Messenger RNA
PMA:	Phorbol 12-myristate 13-acetate
rIL-2:	Recombinant IL-2
RT-PCR:	Reverse transcription-PCR
TcR:	T-cell receptor

5 messenger RNA (mRNA) is increased in the bronchial mucosa of symptomatic asthmatic patients,³ is correlated with the number of activated eosinophils, and increases further on allergen inhalation challenge.^{4,5} Bronchoalveolar lavage fluid from atopic and nonatopic asthmatic patients shows an increased IL-5 concentration.⁶ IL-5 is the predominant eosinophil-active cytokine present in bronchoalveolar lavage fluid obtained from asthmatic patients.⁷ In addition, the serum IL-5 concentration level is elevated in symptomatic asthmatic patients and decreases after oral prednisolone therapy.⁸ Furthermore, several studies in experimental asthma models have revealed that administration of an anti-IL-5 neutralizing antibody inhibits the recruitment of eosinophils into the lung and abrogates the late phase of bronchoconstriction,^{9,10} clearly indicating the essential role of IL-5 in the late-phase asthmatic response. We recently reported that mice adoptively transferred with IL-5-producing T_H clones developed eosinophilic infiltration of the lungs on antigen inhalation in the absence of humoral immunity (including IgG, IgA, or IgE antibodies), indicating the importance of T_H cells as the source of IL-5.¹¹

Our previous studies have shown that IL-5 production by CD4⁺ T cells is enhanced in both atopic and nonatopic asthmatic patients.^{12,13} Thus the control of IL-5 production by activated T cells may be a beneficial approach to the management of eosinophilic inflammation. IL-2 and IL-4 synthesis by T_H cells is mainly controlled by transcriptional regulation,¹⁴⁻¹⁶ so it is important to delineate the molecular mechanisms regulating human IL-5 gene transcription. Several investigators have analyzed the murine IL-5 promoter/enhancer gene. The GATA-3 element and the conserved lymphokine element 0 of the

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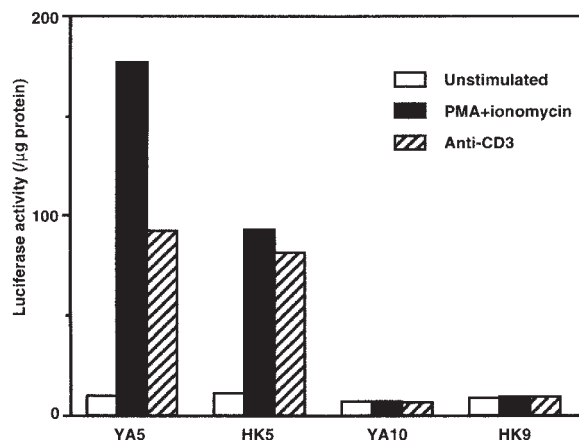


FIG 1. IL-5 transcriptional activity of IL-5-producing and IL-5-non-producing T-cell clones. T-cell clones were transiently transfected with pIL-5(-511)Luc (50 μ g) and pCMV- β -gal (10 μ g) by electroporation (270 V, 960 μ F) with a 0.4-cm cuvette. Cells were either stimulated or left unstimulated in 24-well culture plates as indicated. For TcR stimulation, wells were pretreated overnight at 4°C with OKT3 antibody (10 μ g/mL) in 0.05 mol/L carbonate-bicarbonate buffer (pH 9.6). After 24 hours, cell lysates were prepared and tested for luciferase and β -galactosidase activity. Data are the mean of triplicate cultures.

murine IL-5 enhancer gene are important for the response to cAMP.¹⁷ A positively regulating element (-244 to -81) and negatively regulating elements (-431 to -392 and -300 to -261) have also been located in this gene.¹⁸ The phorbol 12-myristate 13-acetate (PMA) responsive element, designated as IL-5P (-117 to -92), is related to NF-AT binding factors,¹⁹ although TCATTT-binding factor is induced on stimulation and is downregulated by cyclosporin A.²⁰ Some studies have used murine EL4 cells to analyze the human IL-5 gene because of the lack of appropriate human T-cell systems.^{21,22} There has been only 1 study²³ that analyzed the human IL-5 gene with the use of human T cells, although the cells used were transformed by human T-cell leukemia virus type I and constitutively produced high levels of various cytokines. We have also reported that IL-5 gene transcription is induced by IL-2 receptor (IL-2R) signaling, although IL-2 and IL-4 synthesis are not induced.²⁴ Elucidation of the unique transcriptional regulation mechanism of the IL-5 gene might facilitate the development of novel treatments that specifically downregulate IL-5 gene transcription without affecting other T-cell functions.

A major reason for the relative paucity of information on human IL-5 gene transcription is the lack of cell lines that retain inducible IL-5 gene transcription similar to that of peripheral T_H cells and that can be used for transfection analysis. To analyze the cellular and molecular mechanisms of IL-5 synthesis by human CD4⁺ T_H cells, we established allergen-specific T-cell clones and T-cell hybridomas from atopic asthmatic patients. As a result, the unique regulatory mechanism of human T-cell IL-5 gene transcription could be elucidated.

METHODS

Reagents

OKT3, an anti-CD3 mAb, was obtained from Ortho (Raritan, NJ). Anti-CD28 (L293), fluorescein isothiocyanate-labeled anti-CD3 (Leu4), anti-CD4 (Leu2), and anti-CD8 (Leu3) mAbs were obtained from Becton Dickinson (San Jose, Calif). Recombinant Der f 2 (rDer f 2; a major house dust mite allergen) protein was provided by Asahi Breweries Ltd.²⁵ AIM-V medium (Gibco BRL, Gaithersburg, Md) was used for T-cell cultures. RPMI-1640 medium (Gibco BRL) supplemented with 10% FBS, 100 μ g/mL streptomycin, and 100 U/mL penicillin was used for the transfection experiments.

Allergen-specific human T-cell clones and T-cell hybridomas

Der f 2-specific human T-cell clones were obtained from PBMCs of atopic asthma donors by antigenic stimulation followed by the limiting dilution method, as described previously.²⁶ Briefly, PBMCs (2 $\times$ 10⁶/mL) obtained from atopic asthmatic donors were stimulated with rDer f 2 protein (1 μ g/mL) followed by limiting dilution. The resulting T-cell clones were expanded by antigenic stimulation, harvested at least 10 days after the last antigenic stimulation, layered onto Ficoll-Paque (Amersham Pharmacia Biotech AB, Uppsala, Sweden), and centrifuged. The interface was then recovered, washed twice, and resuspended in fresh medium. The resulting preparation usually consisted of more than 98% CD3 positive cells as determined by flow cytometry. T-cell hybridomas were established by fusing IL-5-producing and IL-5-nonproducing T-cell clones with a hypoxanthine guanine phosphoribosyl transferase-deficient T-cell line and were maintained as described previously.²⁷

Stimulation of T-cell clones

T cells were harvested at least 10 days after the last antigenic stimulation, layered onto Ficoll-Paque, and centrifuged. Cells at the interface were recovered, washed twice, and resuspended in fresh medium. The resulting preparation usually consisted of more than 98% CD3-positive cells as determined by flow cytometry. These cells (10⁵ per well) were cultured in triplicate with various stimulants in 96-well round-bottomed culture plates for the designated periods. For proliferation assays, cells were cultured for 72 hours, and ³H-thymidine (0.5 μ Ci per well) was added for the last 16 hours. To obtain cytoplasmic RNA and nuclear extracts, T cells (4 $\times$ 10⁶/well) were cultured in 24-well culture plates with various stimulants for the designated periods.

Quantitation of cytokines in culture supernatants

IL-5 was measured by a sandwich ELISA with monoclonal anti-human IL-5 (D138) as the capture antibody and biotinylated purified rabbit anti-human IL-5 as the second antibody, as described previously.¹³ IL-2 and IL-4 were measured by specific ELISAs (Quantikine ELISA kits; R&D systems, Minneapolis, Minn) according to the manufacturer's directions.

Reverse transcription-PCR assay

IL-5 gene expression was analyzed by reverse transcription-PCR (RT-PCR), as reported previously.¹³ Briefly, RNA was extracted from pelleted cells essentially according to the 1-step acid guanidinium isothiocyanate/phenol-chloroform extraction method of Chomczynski and Sacchi²⁸ with Isogene (Nippongene, Tokyo, Japan). cDNA was synthesized from 1 μ g of cytoplasmic RNA with the use of random primers and murine Moloney leukemia virus reverse transcriptase (Gibco BRL), according to the manufacturer's directions. The PCR was performed with sets of primers specific for

TABLE I. Cytokine production profiles of the Der f 2-specific T-cell clones

Clone	Stimulus	Cytokine production (pg/mL)*		
		IL-2	IL-4	IL-5
YA5	None	<6	<3	<1
	PMA+IOM	1640 ± 69	740 ± 62	410 ± 29
	OKT3†	1320 ± 71	480 ± 39	480 ± 32
HK5	None	<6	<3	<1
	PMA+IOM	1220 ± 112	821 ± 72	692 ± 50
	OKT3	840 ± 72	470 ± 52	724 ± 39
YA10	None	<6	<3	<1
	PMA+IOM	1920 ± 52	840 ± 61	<1
	OKT3	1020 ± 32	940 ± 28	<1
HK9	None	<6	<3	<1
	PMA+IOM	980 ± 49	700 ± 82	<1
	OKT3	860 ± 71	640 ± 42	<1

IOM, ionomycin.

*T-cell clones (10⁵ per well) were cultured in triplicate in 96-well round-bottomed culture plates with various stimulants. The supernatants were collected after 24 hours and assayed for IL-2, IL-4, and IL-5 with specific ELISAs. Data are presented as the mean ± SEM of triplicate cultures. The minimum detectable concentration was 6 pg/mL for IL-2, 3 pg/mL for IL-4, and 1 pg/mL for IL-5.

†Wells were pretreated overnight at 4°C with OKT3 antibody (10 µg/mL) in 0.05 mol/L carbonate-bicarbonate buffer (pH 9.6).

human IL-5 and β-actin (Clontech, Palo Alto, Calif), and PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide staining.

Plasmid constructs

Luciferase reporter plasmids containing the human IL-5 promoter/enhancer gene (−511 to +4 relative to the transcription initiation site), IL-2 promoter/enhancer gene (−480 to +2), and IL-4 promoter/enhancer gene (−270 to +2) were constructed as reported previously²⁹ and were designated as pIL-5(−511)Luc, pIL-2(−480)Luc, and pIL-4(−270)Luc, respectively. The pCMV-β-gal control vector (Riken DNA Bank, Tokyo, Japan) was used as a transfection control.

Transient transfection, luciferase assay, and β-galactosidase assay

T-cell clones and hybridomas were transfected by electroporation with a Gene Pulser (Bio-Rad, Richmond, Calif), as described previously.²⁷

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was performed according to the manufacturer's protocol (Gel Shift Assay Systems; Promega) with slight modifications, as described previously.²⁹ Crude nuclear extracts were prepared from unstimulated or stimulated cells as described by Schreiber et al,³⁰ with some modifications. Pairs of synthetic HPLC-purified oligonucleotides (Sawady Technology, Tokyo, Japan) containing complementary sequences were annealed and end-labeled with ³²P by T4 polynucleotide kinase (Takara, Otsu, Japan). The DNA sequences of each oligonucleotide were as follows: AP-1 (5′-CGCTTGATGAGTCAGC-CGGAA-3′),³¹ Oct-1 (5′-TGTCGAATGCAAATCACTAGAA-3′),³² NF-κB (5′-AGTTGAGGGGACTTTCCAGGC-3′),³³ and NF-AT (5′-GGAGGAAAACTGTTTCATACAGAAGGCGT-3′) from the distal NF-AT site of the human IL-2 gene.³⁴

Protein assay

Protein concentrations were determined with the bicinchoninic acid protein assay reagent (Pierce, Rockford, Ill) according to the manufacturer's directions.

Statistical analysis

Statistical analysis was performed with the Student *t* test, and *P* < .05 was considered statistically significant. Results are presented as the mean ± SEM.

RESULTS

IL-5 synthesis by human T_H cells regulation at the transcriptional level

Der f 2-specific T-cell clones were established from peripheral blood lymphocytes of atopic asthmatic patients, as described previously.^{26,35} All the T-cell clones established were of the T_{H0} phenotype because they produced both IL-2 and IL-4 (data not shown). The cytokine production profiles of the T_H clones used for transfection analysis are shown in Table I. YA5 and HK5 produced significant amounts of IL-2, IL-4, and IL-5 on stimulation, whereas YA10 and HK9 produced equivalent amounts of IL-2 and IL-4, but no detectable IL-5.

IL-5-producing (YA5 and HK5) and IL-5-nonproducing (YA10 and HK9) T-cell clones were transiently transfected with pIL-5(−511)Luc and then either cultured with 20 nmol/L PMA plus 1 µmol/L ionomycin, cultured in wells precoated with OKT3 mAb, or left unstimulated. Immobilized OKT3 mAb, a stimulating anti-CD3 mAb, activates T cells by crosslinking TcR-CD3 complexes, which physiologically transduce activating signals into the cytoplasm. pCMV-β-gal was cotransfected as a transfection control. As shown in Fig 1, pIL-5(−511)Luc was efficiently transcribed on stimulation of YA5 and HK5 cells, indicating that the 515-base pair IL-5 gene segment 5′ upstream of the coding region is functionally sufficient for stimulation-dependent gene transcription in human T_H cells. In contrast, the IL-5-nonproducing T-cell clones (YA10 and HK9) did not transcribe pIL-5(−511)Luc. The transfection efficiency was determined from the β-galactosidase activity derived from the cotransfected control vector (pCMV-β-gal) and was not significantly different among the 4 clones (OD₅₇₄, 0.44 ± 0.08; YA5, 0.51 ± 0.06; HK5, 0.45 ± 0.04; YA10, 0.50 ± 0.08; HK9). pIL-5(−511)Luc was transcribed by IL-5-producing clones, but not by IL-5-nonproducing clones, suggesting that transcriptional control is also important in IL-5 synthesis as is the case in IL-2 synthesis.

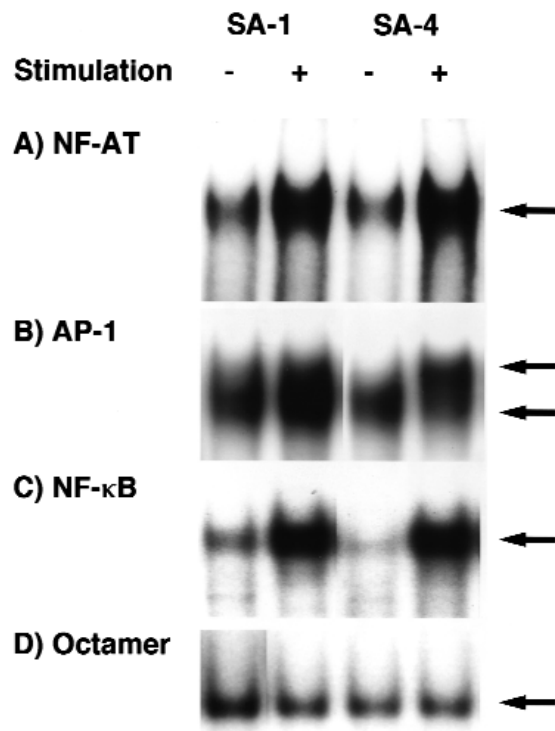


FIG 2. AP-1, NF-AT, NF-κB, and octamer binding activities of IL-5 gene-expressing and -nonexpressing T-cell hybridomas. CEM-8Az, SA-1, and SA-4 cells were stimulated with PMA (20 nmol/L) plus ionomycin (1 μmol/L) or were left unstimulated for 2 hours. Nuclear protein was then extracted and DNA binding activity was measured by EMSA. The bands (arrows) represent specific binding determined by the addition of an excess of unlabeled probe in the preliminary experiments.

Transcriptional regulation of IL-2, IL-4, and IL-5 genes in IL-5 gene-expressing and -nonexpressing T-cell hybridomas

To facilitate the analysis of IL-5 gene transcription, human T-cell hybridomas were constructed by fusing allergen-specific T-cell clones with an 8-azaguanine-resistant human transformed T-cell line (CEM-8Az).²⁷ An IL-5-producing T-cell clone was fused with CEM-8Az to construct SA-1 cells that expressed IL-5 mRNA on activation. In contrast, SA-4 cells derived from fusion between an IL-5-nonproducing T-cell clone and CEM-8Az failed to express IL-5 mRNA.

The transcriptional activity of the IL-2, IL-4, and IL-5 genes was next compared among SA-1, SA-4, CEM-8Az, and Jurkat cells (Table II). Cells transiently transfected with pIL-2(-480)Luc, pIL-4(-270)Luc, or pIL-5(-511)Luc were stimulated with PMA (20 nmol/L) plus ionomycin (1 μmol/L). pIL-2(-480)Luc and pIL-4(-270)Luc was transcribed on stimulation of all 4 cell lines. Strikingly, there was a 27-fold increase of luciferase activity on stimulation of SA-1 cells transfected with pIL-5(-511)Luc, although the luciferase activity induced in SA-4, CEM-

TABLE II. Transcriptional activity of cytokine genes induced in T-cell hybridomas

Cell line*	Relative fold induction of luciferase activity†		
	pIL-2(-480)Luc	pIL-4(-270)Luc	pIL-5(-511)Luc
CEM-8Az	3.5	2.8	1.0
SA-1	25.1	15.5	27.9
SA-4	33.9	14.5	1.0
Jurkat	52.2	7.9	1.0

*Cells (5×10^6 in 500 μL of RPMI-1640 medium) were transiently transfected with pIL-2(-480)Luc, pIL-4(-270)Luc, or pIL-5(-511)Luc by electroporation (250 V; 960 μF) with a 0.4-cm cuvette and were cultured in triplicate with PMA (20 nmol/L) plus ionomycin (1 μmol/L) or were left unstimulated for 24 hours.

†Cell lysates were prepared and tested for the luciferase and β-galactosidase activities. Protein concentrations were determined with bicinchoninic acid protein assay reagent. Fold induction was calculated as the induced relative luciferase unit divided by the uninduced relative luciferase unit. Relative fold induction was calculated as the fold induction of each transfected plasmid divided by the fold induction of the promoterless pGL2 basic vector. Data are the mean of triplicate cultures.

8Az, and Jurkat cells transfected with pIL-5(-511)Luc was not significantly different from that in cells transfected with a promoterless pGL2 basic vector. Gene transcription mediated by the 515-bp human IL-5 promoter/enhancer segment was only initiated by the IL-5 gene-expressing hybridoma and not by the IL-5 gene-nonexpressing hybridoma, CEM-8Az, or Jurkat, results that were consistent with the transcriptional regulation data obtained with T-cell clones. Similar results were obtained with 3 other IL-5 gene-expressing hybridomas and 4 IL-5 gene-nonexpressing hybridomas (data not shown).

AP-1, NF-AT, NF-κB, and octamer binding activity in IL-5 gene-expressing and -nonexpressing T-cell hybridomas

The AP-1, NF-AT, NF-κB, and Oct-OAP⁴⁰ binding elements have a crucial role in IL-2 gene transcription,¹⁵ although AP-1 and NF-AT also are involved in IL-4 gene transcription.³⁶ EMSA was performed to compare the expression of these transcription factors by our hybridomas. A representative result is shown in Fig 2. SA-1 and SA-4 cells were stimulated with PMA (20 nmol/L) plus ionomycin (1 μmol/L) or left unstimulated for 2 hours, and then nuclear extracts were prepared. NF-AT, AP-1, and NF-κB binding activities were induced on activation, and octamer-binding activity was constitutively expressed in both SA-1 and SA-4 cells. Essentially the same results were obtained with nuclear proteins prepared from the IL-5-producing and -nonproducing T-cell clones (data not shown).

IL-2 signal initiated IL-5 gene transcription without NF-AT, AP-1, or NF-κB induction

These findings indicated that IL-5 synthesis in human T_H cells may be regulated by a transcriptional mechanism distinct from those regulating IL-2 and IL-4 synthesis.

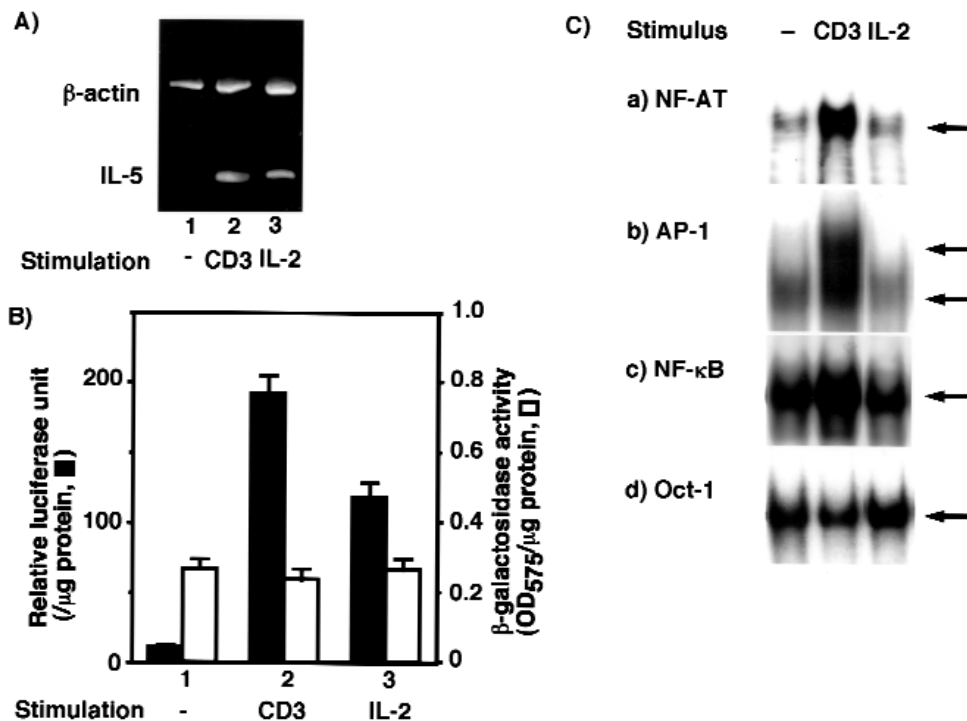


FIG 3. IL-5 production induced by IL-2. **A**, T-cell clones (YA5; 2×10^6 per well) were stimulated with rIL-2 (100 U/mL) or immobilized OKT3 mAb for 8 hours. Total RNA was extracted, reverse transcribed, and amplified by the PCR. The 279- and 1128-bp products corresponded with the expected size of IL-5 and β -actin amplification products, respectively. **B**, T-cell clones (YA5; 2.5×10^7) were transiently transfected with pIL-5Luc(-511) and pCMV- β -gal by electroporation. Cells were incubated with or without rIL-2 (100 U/mL) for 24 hours. For TcR stimulation, cells were incubated in wells coated with OKT3 mAb (10 μ g/mL). Cell lysates were assayed for luciferase (black bar) and β -galactosidase activity (white bar). Data are the mean $\pm$ SEM of triplicate cultures. **C**, T-cell clones (YA5; 2×10^6 per well) were stimulated with immobilized anti-CD3 mAb or rIL-2 (100 U/mL) for 2 hours. Nuclear protein was then extracted, and DNA binding activity was measured by EMSA. The bands (arrows) represent specific binding determined by the addition of an excess of unlabeled probe in preliminary experiments.

Such unique transcriptional regulation was further substantiated by the following findings: The T-cell clones that produced IL-5 on antigenic stimulation also produced IL-5 in response to exogenous IL-2.²⁴ IL-5 synthesis mediated by the IL-2R was then further investigated. It was found that IL-2-induced IL-5 production was accompanied by marked upregulation of IL-5 mRNA (Fig 3, A). The peak level of IL-5 mRNA induced by IL-2 was equivalent to that induced by immobilized anti-CD3 (Fig 3, A) and PMA plus ionomycin (data not shown).

Transfection of T-cell clones with pIL-5(-511)Luc followed by stimulation with recombinant IL-2 (rIL-2; 100 U/mL) resulted in more than an 80-fold increase in luciferase activity compared with that of the unstimulated cells (Fig 3, B), whereas β -galactosidase activity derived from pCMV- β -gal did not differ significantly between stimulated and unstimulated T-cell clones. In contrast, pIL-4(-270)Luc was not transcribed in response to IL-2 (data not shown), a finding corresponding with the absence of IL-4 protein production.

Possible involvement of transcription factors such as NF-AT, AP-1, NF- κ B, and Oct-1 in the IL-2-induced IL-5 synthesis was next analyzed by EMSA (Fig 3, C). On

stimulation with immobilized anti-CD3 mAb, the binding activities of NF-AT, AP-1, and NF- κ B were markedly induced in the nuclear protein preparations. Oct-1 binding activity was constitutively present in both stimulated and unstimulated cells. In contrast to TcR-stimulation, neither NF-AT, AP-1, nor NF- κ B were upregulated in the nuclear extracts of IL-2-stimulated T cells. Essentially the same results were obtained with 3 other T-cell clones (data not shown).

DISCUSSION

Our present findings clearly indicate that (1) IL-5 synthesis by human T cells is regulated at the transcriptional level and (2) IL-5 synthesis by human T_H clones is induced independently from IL-2 or IL-4 synthesis. Our results also suggested that a unique transcription factor other than AP-1, NF-AT, NF- κ B, or Oct-1 is critical for the regulation of IL-5 gene in human T_H cells.

The relationship between IL-5 protein production and gene transcription was investigated first. IL-5 protein production by allergen-specific T_H clones was associated with inducible transcriptional activity of the IL-5 gene,

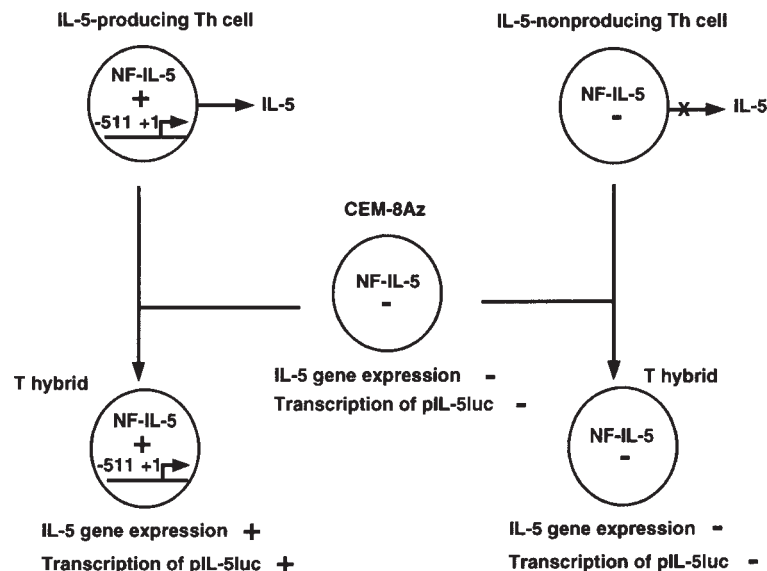


FIG 4. A positive regulatory factor may operate through the approximately 500-bp IL-5 promoter/enhancer gene. An IL-5 gene-expressing T-cell clone was fused with CEM-8Az cells to construct an IL-5 gene-expressing hybridoma that was capable of transcribing pIL-5(-511)Luc. An IL-5 gene-nonexpressing T-cell clone was fused with CEM-8Az cells to construct an IL-5 gene-nonexpressing hybridoma that was incapable of transcribing pIL-5(-511)Luc. The results suggested that IL-5 synthesis is regulated by a mechanism that positively influences the IL-5 gene, rather than by a repressor mechanism. The responsible transcription factor seemed to operate through the approximately 500-bp promoter/enhancer region contained in pIL-5(-511)Luc.

as determined by the transient transfection of pIL-5(-511)Luc followed by stimulation of the cells (Fig 1). T_H cells that did not transcribe pIL-5(-511)Luc on stimulation were incapable of synthesizing IL-5 protein. These results strongly suggested that IL-5 synthesis by human T_H cells was regulated at the transcriptional level, as is the case for IL-2 synthesis.¹⁵

The information on human IL-5 gene transcription is still limited, although quite a few studies have been performed on the murine IL-5 gene.^{17-20,37,38} Several authors have even used transformed murine T cells to analyze the human IL-5 gene.^{21,22} To investigate human IL-5 gene transcription, we constructed immortalized human T cells capable of transcribing the human IL-5 gene in an inducible manner. We found that a T-cell hybridoma derived from the fusion of an IL-5-producing T-cell clone with a T-cell tumor line expressed IL-5 mRNA and transcribed pIL-5(-511)Luc on stimulation, whereas another T-cell hybridoma derived from an IL-5-nonproducing T-cell clone did not (Table II), closely reflecting the transcriptional regulation of untransformed T cells. The results obtained with hybridomas strongly suggested a role of transcription factor(s) that positively regulate the approximately 500-bp promoter/enhancer region of the human IL-5 gene rather than involvement of negative repressor(s) (Fig 4). In other words, cells capable of transcribing pIL-5(-511)Luc may express a transcription factor required for IL-5 gene transcription (tentatively named NF-IL-5), although cells incapable of transcribing pIL-5(-511)Luc may not have expressed this factor. It is further suggested that the 515-bp segment used in our study

contains the key element(s) regulating IL-5 gene.

pIL-2(-480)Luc and pIL-4(-270)Luc were transcribed by both SA-1 and SA-4 cells, although pIL-5(-511)Luc was only transcribed by SA-1 cells (Table II), clearly indicating that IL-5 gene transcription is differentially regulated from that of IL-2 and IL-4 by these human T cells. Jurkat cells have been widely used for studying human IL-2 and IL-4 production.¹⁵ Jurkat cells transcribed pIL-2(-480)Luc on activation, indicating that the activating signal was sufficient for IL-2 gene transcription (Table II). Among various transcriptional elements identified in the human IL-2 gene, NF-AT, AP-1, NF-κB, and Oct-OAP⁴⁰ are essential for gene induction.¹⁵ Our results indicated that NF-AT, AP-1, NF-κB, and Oct-1 were not sufficient for IL-5 gene transcription and suggested the involvement of additional transcription factor(s).

Expression of nuclear transcription factors in the IL-5 gene-expressing and -nonexpressing hybridomas was examined next. In both IL-5 gene-expressing and -nonexpressing hybridomas, NF-AT, AP-1, and NF-κB binding were clearly induced on stimulation, whereas Oct-1 binding was constitutively expressed at a stable level (Fig 2), which indicated that the transcription factors regulating the IL-2 gene are present in IL-5 gene-nonexpressing hybridomas and that other factor(s) may be required for IL-5 gene transcription. This unique transcriptional regulation of the IL-5 gene was further substantiated by the following findings.

T-cell clones capable of producing IL-5 on antigenic stimulation also produced IL-5 in response to exogenous IL-2.²⁴ The finding that rIL-2 induced IL-5 production

by cloned T cells clearly indicates that IL-2 directly stimulated T cells to produce IL-5, as essentially pure T cells (>98% were CD3⁺CD4⁺ cells by fluorescence-activated cell sorter analysis) were used in our study. Although the T-cell clones were capable of producing IL-4 on stimulation, IL-4 was never detected in the culture supernatants of T cells stimulated with rIL-2 at any concentration tested (data not shown), indicating that production of IL-4 and IL-5 was differentially regulated by these human T_H cells. rIL-15 also induced IL-5 synthesis with a similar concentration-response relationship to rIL-2, although rIL-4 at concentrations up to 10⁴ U/mL did not induce IL-5 production, suggesting involvement of the IL-2R β and IL-2R γ chains shared between IL-2 and IL-15 signaling.²⁶ The rapid induction of IL-5 mRNA after IL-2 stimulation made it more likely that IL-2 directly activated the IL-5 gene, rather than that IL-2 induced the production of another cytokine by T cells that eventually activated the IL-5 gene. These findings clearly indicate that the approximately 500-bp promoter/enhancer segment of the human IL-5 gene located 5' upstream of the coding region contains IL-2-responsive element(s) that drive the IL-5 gene but not the IL-4 or IL-2 genes.

Fig 3, C, shows that binding of NF-AT, AP-1, and NF- κ B was induced after TcR stimulation but was not apparently upregulated after IL-2R stimulation, despite the fact that the endogenous IL-5 gene expression (Fig 3, A) and transcriptional activity of pIL-5(-511)Luc (Fig 3, B) induced by TcR and IL-2R stimulation were quite comparable. As FK506 suppresses IL-5 synthesis and induction of NF-AT in TcR-stimulated T_H clones,²⁹ NF-AT activity seems to be important for IL-5 synthesis induced by TcR stimulation but is not essential for that induced by IL-2R stimulation. AP-1 and NF-AT are the critical transcription factors for induction of the IL-2 gene; however, these findings suggest that some yet undefined transcription factor(s) distinct from AP-1, NF-AT, NF- κ B, or Oct-1 is vital for the IL-5 gene, because IL-5 gene transcription by human T_H cells occurred without induction of AP-1 or NF-AT. Such transcription factor might activate the human IL-5 gene in allergic diseases associated with eosinophilic inflammation, such as asthma and atopic dermatitis. Identification of the IL-2-responsive element in the IL-5 enhancer should improve our understanding of the molecular mechanisms of human T-cell IL-5 synthesis and may facilitate future treatment of severe atopic diseases by the development of selective IL-5 synthesis inhibitors.

Glucocorticoids have long been considered the most effective treatment for eosinophilic disorders, including chronic asthma.³⁹ The efficacy of glucocorticoids is ascribed to the multiple pharmacologic actions, 1 of which is the suppression of inflammatory cytokine production.^{40,41} We and others^{13,42,43} have reported that IL-5 synthesis by peripheral T_H cells was inhibited by glucocorticoids in vitro. However, glucocorticoids have a wide range of actions not only on the immune system but also on various other tissues and organs, leading to multiple side-effects including hypertension, diabetes mellitus, and osteoporosis. The immunosuppressants, FK506

and cyclosporin A, were recently found to be highly effective for treating severe atopic dermatitis.^{44,45} These agents have a more restricted target specificity compared with glucocorticoids but still interfere with too many T-cell functions, thereby causing generalized immunosuppression. An agent capable of blocking NF-IL-5 activity could selectively suppress IL-5 gene transcription without affecting that of IL-2 or IL-4 and would provide an ideal therapeutic mode to control eosinophilic inflammation without side-effects including immunosuppression.

Our findings indicate that IL-5 synthesis by human T cells is regulated by a unique transcription factor distinct from those regulating the IL-2 and IL-4 genes. Such a molecule may be critically involved in eosinophilic inflammation in vivo. Identification of NF-IL-5 should improve our understanding of the pathogenesis of allergic diseases and facilitate the development of selective inhibitors of IL-5 synthesis. Development of a "pinpoint" gene transcription inhibitor may open up a new era in the treatment of human disease.

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